Development of Aptamers to Waterborne Parasites

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The Safe Drinking Water Act Amendment of 1996 mandates that the U. S. Environmental Protection Agency (EPA) evaluate public health risks associated with drinking water contaminants to include waterborne parasites, such as *Cryptosporidium* and *Giardia*. Additionally, the Agency establishes a list of unregulated microbiological contaminants that have potential pathogenic transmission via water. This list is referred to as the Candidate Contaminant List (CCL). Although free-living stages of protozoans are generally larger microorganisms that are susceptible to filtration and sedimentation, many protozoans have spore or cyst stages that are not susceptible to filtration and/or disinfection. Therefore, the detection of pathogenic parasites is essential for maintaining safe drinking water.

Detection of waterborne parasites is primarily based on antibody-antigen reaction assays that vary in sensitivity and specificity. Also, antigenic cross-reactivity between pathogenic and non-pathogenic species represents a problem when exclusively using these assays to monitor safe drinking water. Our goal is to provide an improved detection system based on a new technology termed "Systematic Evolution of Ligands by Exponential enrichment" (SELEX). The final products of SELEX are short, single-stranded oligonucleotides termed "aptamers," which form secondary structures that exhibit high affinity to targeted proteins and/or organisms. In this study, the SELEX process is being employed in the development of an aptamer to the EPA-regulated pathogenic parasite, *Giardia lamblia.* Briefly, the development of a specific aptamer begins with a large combinatorial library of randomized sequences that is mixed with *G. lamblia*. Aptamers that have bound *Giardia* cysts are selected and amplified via the polymerase chain reaction (PCR) for further enrichment cycles. Candidate aptamers will be cloned and sequenced. These sequences will be evaluated for similar motifs and structures for potential assay development utilizing fluorochromes and/or immunomagnetic detection systems. Aptamers offer advantages over traditional antibody (protein)-based systems in cost and flexibility of assay platforms, s well as in sensitivity and specificity. Once developed, this technique will allow the EPA to develop more sensitive and specific methods to detect pathogenic parasites in environmental waters.